

Patent claims

1. Nucleic acid, which comprises at least one region
coding for an enzyme activity which is involved in
5 biosynthesis of spinosyns.
2. Nucleic acid according to Claim 1, characterized
in that it is a single-stranded or double-stranded
DNA or RNA.
- 10 3. Nucleic acid according to Claim 2, characterized
in that it is a DNA fragment.
4. Nucleic acid according to Claim 3, characterized
15 in that it comprises all regions coding for enzyme
activities which are involved in biosynthesis of
spinosyns.
5. Nucleic acid according to any of Claims 1 to 4,
20 characterized in that the enzyme activities are of
polyketide synthases, methyltransferases,
glycosyltransferases, epimerases, amino-
transferases, dimethyltransferases, reductases,
dehydratases and/or cyclization enzymes.
- 25 6. Nucleic acid according to any of Claims 1 to 5,
characterized in that it originates from
Saccharopolyspora spinosa.
- 30 7. Nucleic acid according to Claim 1, comprising at
least one sequence selected from
 - (a) the sequences according to SEQ ID NOS: 1, 2,
3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19, 21, 23,
35 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45,
47, 49, 51, 52 or 54,

- (b) part sequences of at least 14 base pairs in length of the sequences defined under (a),
- (c) sequences which hybridize to the sequences defined under (a),
- (d) sequences which are at least 70% identical to the sequences defined under (a),
- (e) sequences which are complementary to the sequences defined under (a), and
- (g) sequences which, due to the degeneracy of the genetic code, code for the same amino acid sequence as the sequences defined under (a) to (d).
8. Nucleic acid according to Claim 7, characterized in that it comprises the sequence according to SEQ ID NOS: 1 to 6.
9. Nucleic acid according to Claim 7, characterized in that it comprises the sequence according to SEQ ID NO: 4.
10. Nucleic acid according to Claim 7, characterized in that it comprises the sequence according to SEQ ID NOS: 5 and 6.
11. Nucleic acid according to Claim 7, characterized in that it comprises at least one sequence according to SEQ ID NOS: 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39.
12. Nucleic acid according to Claim 7, characterized in that it comprises at least one sequence according to SEQ ID NOS: 41, 43, 45, 47 or 49.

13. Regulatory region, which controls transcription of a nucleic acid according to any of Claims 1 to 7 in *Saccharopolyspora spinosa*.
- 5 14. DNA construct comprising a nucleic acid according to any of Claims 1 to 12 and at least one heterologous promoter.
- 10 15. Vector comprising at least one nucleic acid according to any of Claims 1 to 12, a regulatory region according to Claim 13 or a DNA construct according to Claim 14.
- 15 16. Vector according to Claim 15, characterized in that the nucleic acid is functionally linked to regulatory sequences which ensure expression of the coding regions of the nucleic acid in prokaryotic or eukaryotic cells.
- 20 17. Vector according to either of Claims 15 and 16, characterized in that it is a BAC vector, PAC vector or a vector functionally equivalent to BAC or PAC vectors.
- 25 18. Vector according to Claim 17, characterized in that it is a vector corresponding to the BAC clones having the deposition numbers DSM 13010, DSM 13011 or DSM 13012.
- 30 19. Vector according to any of Claims 15 to 18, characterized in that it is a shuttle vector which can be transferred both to prokaryotes and to eukaryotes.
- 35 20. Vector according to any of Claims 15 to 19, characterized in that it is a shuttle vector which can be transferred both to Gram-negative and Gram-positive bacteria and to Archea.

21. Vector according to any of Claims 15 to 19,
characterized in that it is a shuttle vector which
can be transferred both to *Escherichia coli* and to
actinomycetes.
22. Vector according to Claim 21, characterized in
that it is a shuttle vector which can be
transferred both to *Escherichia coli* and to
Streptomyces.
23. Vector according to any of Claims 15 to 22,
characterized in that it can be replicated
autonomously in a prokaryote.
24. Vector according to any of Claims 15 to 22,
characterized in that it can be integrated into
the genome of a prokaryote under involvement of
the phage Φ C31 integration mechanism, the pSAM2
integration mechanism or the mini-circle
integration mechanism.
25. Vector according to any of Claims 15 to 22,
characterized in that it can be integrated into
the genome of a prokaryote by RecA-mediated
recombination.
26. Vector according to any of Claims 15 to 22,
characterized in that it can be integrated into
the genome of a prokaryote by RecE- and RecT-
mediated recombination.
27. Host cell comprising a nucleic acid according to
any of Claims 1 to 12, a regulatory region
according to Claim 13, a DNA construct according
to Claim 14 or at least a vector according to any
of Claims 15 to 26.

28. Host cell according to Claim 27, characterized in that it is a prokaryotic or eukaryotic cell.
- 5 29. Host cell according to Claim 28, characterized in that the prokaryotic cell belongs to the group of actinomycetes, preferably the group of streptomycetes.
- 10 30. Host cell according to Claim 28, characterized in that the eukaryotic cell is a plant cell.
31. Polypeptide which is encoded by a nucleic acid according to any of Claims 1 to 7.
- 15 32. Polypeptide according to Claim 31, characterized in that it has a methyltransferase activity.
33. Polypeptide according to Claim 32, characterized in that it has the amino acid sequence according to SEQ ID NOS: 8, 12, 14, 18 or 20, or a part sequence thereof.
- 20 34. Polypeptide according to Claim 31, characterized in that it has a glycosyltransferase activity.
- 25 35. Polypeptide according to Claim 34, characterized in that it has the amino acid sequence according to SEQ ID NOS: 10 or 30, or a part sequence thereof.
- 30 36. Polypeptide according to Claim 31, characterized in that it has the activity of a C-C linking enzyme which carries out cyclization reactions.
- 35 37. Polypeptide according to Claim 36, characterized in that it has the amino acid sequence according to SEQ ID NO: 16 or a part sequence thereof.

38. Polypeptide according to Claim 31, characterized in that it has the activity of an enzyme which is involved in cyclization reactions.
- 5 39. Polypeptide according to Claim 38, characterized in that it has the amino acid sequence according to SEQ ID NO: 22 or a part sequence thereof.
- 10 40. Polypeptide according to Claim 31, characterized in that it has a 2,3-reductase activity.
41. Polypeptide according to Claim 40, characterized in that it has the amino acid sequence according to SEQ ID NO: 24 or a part sequence thereof.
- 15 42. Polypeptide according to Claim 31, characterized in that it has a 2,3-dehydratase activity.
- 20 43. Polypeptide according to Claim 42, characterized in that it has the amino acid sequence according to SEQ ID NO: 26 or a part sequence thereof.
44. Polypeptide according to Claim 31, characterized in that it has a thioesterase activity.
- 25 45. Polypeptide according to Claim 44, characterized in that it has the amino acid sequence according to SEQ ID NO: 28 or a part sequence thereof.
- 30 46. Polypeptide according to Claim 31, characterized in that it has a 3,4-dehydratase activity.
47. Polypeptide according to Claim 46, characterized in that it has the amino acid sequence according to SEQ ID NO: 32 or a part sequence thereof.
- 35 48. Polypeptide according to Claim 31, characterized in that it has a 4-aminotransferase activity.

49. Polypeptide according to Claim 48, characterized in that it has the amino acid sequence according to SEQ ID NO: 34 or a part sequence thereof.
- 5 50. Polypeptide according to Claim 31, characterized in that it has an N-dimethyltransferase activity.
- 10 51. Polypeptide according to Claim 50, characterized in that it has the amino acid sequence according to SEQ ID NO: 36 or a part sequence thereof.
- 15 52. Polypeptide according to Claim 31, characterized in that it has a 3,4-reductase activity.
- 20 53. Polypeptide according to Claim 52, characterized in that it has the amino acid sequence according to SEQ ID NO: 38 or a part sequence thereof.
- 25 54. Polypeptide according to Claim 31, characterized in that it has a transcription regulator activity.
55. Polypeptide according to Claim 54, characterized in that it has the amino acid sequence according to SEQ ID NO: 40 or a part sequence thereof.
- 30 56. Polypeptide according to Claim 31, characterized in that it has a polyketide synthase activity.
- 35 57. Polypeptide according to Claim 56, characterized in that it has the amino acid sequence according to SEQ ID NOS: 42, 44, 46, 48 or 50, or a part sequence thereof.
58. Polypeptide according to Claim 31, characterized in that it has a glucose dehydratase activity.

59. Polypeptide according to Claim 58, characterized in that it has the amino acid sequence according to SEQ ID NO: 53.
- 5 60. Polypeptide according to Claim 31, characterized in that it has a 3,5-epimerase activity.
61. Polypeptide according to Claim 60, characterized in that it has the amino acid sequence according to SEQ ID NO: 55.
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62. Enzymes which are involved in cyclization reactions, characterized in that they comprise the amino acid sequence according to SEQ ID NO: 15 or 22 or a part sequence thereof which is still able to carry out at least part of the reaction or in that they are at least 50% identical thereto at the amino acid level.
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63. Antibody, which reacts specifically with a polypeptide according to any of Claims 31 to 62.
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64. Method for preparing a nucleic acid according to any of Claims 1 to 7, comprising the following steps:
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- (a) complete chemical synthesis in a manner known per se or
- (b) chemical synthesis of oligonucleotides, labelling of the oligonucleotides, hybridizing the oligonucleotides to DNA of a genomic or cDNA library which has been prepared by starting from genomic DNA or mRNA from *S. spinosa*, selecting positive clones and isolating the hybridizing DNA from positive clones or
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- (c) chemical synthesis of oligonucleotides and amplification of the target DNA by means of PCR.

5 65. Method for preparing a polypeptide according to any of Claims 31 to 62, comprising the following steps:

10 (a) culturing a host cell according to any of Claims 27 to 30 under conditions which ensure expression of the nucleic acid according to any of Claims 1 to 7, or

15 (a1) expressing a nucleic acid according to any of Claims 1 to 7 in an *in vitro* system, and

(b) obtaining the polypeptide from the cell, the culture medium or the *in vitro* system.

20 66. Method for preparing spinosyn, spinosyn precursors or spinosyn derivatives, comprising the following steps:

25 (a) culturing a host cell according to any of Claims 27 to 30 under conditions which ensure expression of the nucleic acid according to any of Claims 1 to 7, and

30 (b) obtaining the spinosyn, spinosyn precursor or spinosyn derivative from the cell or the culture medium.

67. Method for preparing spinosyn derivatives, including spinosyn precursors, comprising the following steps:

(a) exchanging at least one module-encoding nucleic acid sequence according to Claim 7

for at least one other module-encoding nucleic acid sequence according to Claim 7, or

5 (b) exchanging at least one module-encoding nucleic acid sequence according to Claim 7 for at least one other module-encoding nucleic acid sequence from *S. spinosa*, or

10 (c) exchanging at least one module-encoding nucleic acid sequence according to Claim 7 for at least one other module-encoding nucleic acid sequence from an organism other than *S. spinosa*, or

15 (d) exchanging at least one domain-encoding nucleic acid sequence according to Claim 7 for at least one other domain-encoding nucleic acid sequence according to Claim 7, or
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(e) exchanging at least one domain-encoding nucleic acid sequence according to Claim 7 for at least one other domain-encoding nucleic acid sequence from *S. spinosa*, or
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(f) exchanging at least one domain-encoding nucleic acid sequence according to Claim 7 for at least one other domain-encoding nucleic acid sequence from an organism other than *S. spinosa*, or
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(g) exchanging a first acyltransferase-encoding nucleic acid sequence according to Claim 7 for a second acyltransferase-encoding nucleic acid sequence according to Claim 7, wherein the second acyltransferase has a substrate
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specificity different from that of the first acyltransferase, or

5 (h) exchanging a first acyltransferase-encoding nucleic acid sequence according to Claim 7 for a second acyltransferase-encoding nucleic acid sequence from *S. spinosa*, wherein the second acyltransferase has a substrate specificity different from that of the first
10 acyltransferase, or

(i) exchanging a first acyltransferase-encoding nucleic acid sequence according to Claim 7 for a second acyltransferase-encoding nucleic
15 acid sequence from an organism other than *S. spinosa*, wherein the second acyltransferase has a substrate specificity different from that of the first acyltransferase, or

20 (j) deleting at least one domain-encoding nucleic acid sequence according to Claim 7, or

(k) integrating at least one domain-encoding
25 nucleic acid sequence according to Claim 7 into a module-encoding nucleic acid sequence according to Claim 7, or

(l) mutating at least one domain-encoding nucleic
30 acid sequence according to Claim 7,

and expressing the recombinant nucleic acid sequence in a host cell under conditions which allow synthesis of a spinosyn derivative or a
35 spinosyn precursor.

68. Use of a nucleic acid according to any of Claims 1 to 7 for identifying, inactivating and/or modifying spinosyn biosynthesis genes.
- 5 69. Use of a nucleic acid according to any of Claims 1 to 7 for generating a library of polyketide synthases.
- 10 70. Method for attaching a forosamine sugar residue to the spinosyn aglycone or to the spinosyn 17-pseudoaglycone or to a polyketide aglycone, comprising the following steps:
- 15 (a) transferring a nucleic acid according to SEQ ID NOS: 23, 25, 29, 31, 33, 35 and 37 into a host cell which can produce the spinosyn aglycone or the spinosyn 17-pseudoaglycone or the polyketide aglycone, or
- 20 (a1) transferring a nucleic acid according to SEQ ID NOS: 23, 25, 29, 31, 33, 35 and 37 into a host cell which cannot produce the spinosyn aglycone or the spinosyn 17-pseudoaglycone or the polyketide aglycone and adding the
- 25 spinosyn aglycone or the spinosyn 17-pseudoaglycone or the polyketide aglycone to the culture medium, and
- 30 (b) culturing the host cell under conditions which lead to an active cell metabolism.
71. Method for attaching a trimethylrhamnose sugar residue to the spinosyn aglycone or the spinosyn 9-pseudoaglycone or to a polyketide aglycone, comprising the following steps:
- 35 (a) transferring a nucleic acid according to SEQ ID NO: 7, 9, 11, 13, 17 and/or 19 into a host

cell which can produce the spinosyn aglycone or the spinosyn 9-pseudoaglycone or the polyketide aglycone, or

- 5 (a1) transferring a nucleic acid according to SEQ ID NO: 7, 9, 11, 13, 17 and/or 19 into a host cell which cannot produce the spinosyn aglycone or the spinosyn 9-pseudoaglycone or the polyketide aglycone and adding the
10 spinosyn aglycone or the spinosyn 9-pseudoaglycone or the polyketide aglycone to the culture medium, and
- (b) culturing the host cell under conditions
15 which lead to an active cell metabolism.

72. Method according to Claim 71, characterized in that in step (a) nucleic acids according to SEQ ID NOS: 9, 11, 13 and 17 are transferred.